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Abstract

Enumeration of enterococci (EN) bacteria in water is an USEPA approved indicator of fecal pollution and the possible presence of enteric pathogens. Along the northern Gulf of Mexico, the water is shallow with a high organic and particulate load because of the Mississippi River discharge. Disturbance of coastal sediments during wind/wave action caused either by the weather or human activities may increase bacterial counts as a result of increased EN persistence in the water column and/or resuspension of EN in the sediment. The goals of this project are to determine the relationship between organic content and EN counts in the water and whether bacterial resuspension from the sediment contributes to elevated EN counts. We found that EN counts in the water were correlated with wave conditions at seven sites along the Mississippi Gulf Coast. During calm wave conditions, low bacterial levels (1.0 – 227 CFU/100mL) were observed in the water with higher counts in the sediment; the reverse was observed (10 – 351 CFU/100mL) during rough wave conditions. EN counts were positively correlated with organic content of the sediment. Wave activity to keep EN in suspension was apparently critical for high counts. EN counts decreased by 50% in 4 hr from 38 to 17 CFU/100mL in the absence of resuspension and decreased to 1 CFU/100mL after 48 hr. EN in the sediment are not stationary as genetic fingerprinting using REP-PCR showed low persistence of specific isolates over time. Jackknife analysis revealed low similarity among EN isolates from the water and sediment collected on the same day and site during calm wave conditions. This shows that EN are not persisting for long periods in the same area but instead are resuspended and redistributed along the coast. Results from this study provide evidence that high organic content and resuspension of isolates from the sediment during periods of strong wave action contribute to high EN counts. Current research on the survival of EN in estuarine habitats will provide insight on the balance between environmental persistence and fecal pollution in causing high EN counts along beaches in the northern Gulf of Mexico.

Introduction

Enterococci are defined as Gram (+), catalase (-), non spore-forming facultative cocci able to grow at 41ºC. Regulatory agencies use enterococcal counts as the standard for monitoring fecal pollution levels in coastal recreational water. Ocean sediments may serve as a reservoir for enterococci and other indicator bacteria; environmental scientists often suggest that microorganisms have a better change of survival in sediment than in the overlying water. Furthermore, human pathogens and fecal indicators are thought to persist in the environment by attaching to biofilms, clays or algae, and by entering into the viable but non-cultivable state.

Previous work has shown that the enterococci are found in both the water and the fluffy sediment and researchers have examined the significance of sediments, marsh, and seaweed as sources of enterococci in water. Bacteria reattach to particles in the water column, become part of the sediment surface, and can reenter the water column in the attached state. Enterococci in/on sediment are protected from UV inactivation, bacteriophage or toxins and sediments may also provide nutrients that originate from algae, plankton, and other organic debris.

Enterococci's ability to survive for long periods in sediment may be due to desiccation. This occurs when sediments are exposed to fecal contamination from high tides or storm flow events. After the water level drops, the exposed sediment dries and the bacteria become desiccated. Enterococci are known to survive for weeks in the desiccated state in both salt and fresh water and growth can occur once the sediment is rewetted.

The organic content in the sediment may also play a protective role in bacterial counts in sediment. Sediment with elevated levels of organic matter generally have a higher bacterial level than those with low organic content.

The predominant enterococcal species found in the sediment and in water are *Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus,* and *Enterococcus mundtii.* Enterococci may be more abundant in marine sediment than other indicator organisms, including *E. coli,* and they maybe able to grow at a wider range of temperature (10 - 45oC), pH (4.8-9.6), are more resilient in seawater, and are not easily inactivated by sunlight.

Objectives of this study

To determine if the levels of organic material in the sediment correlate with the levels of enterococci in sediment.
To determine how long sediment enterococci stay suspended in the water column after it is disturbed.
To determine if enterococci found in the water column produce fingerprints that positively correlate with beach sediment isolate fingerprints.

Materials and Methods

Mississippi Department of Environmental Quality sampling sites 7A, 8, 9,10,11 and 12A were tested during this study (Figure 1). Water (20, 40, and 80mL) and sediment (15, 25, 50mL) samples were filtered through a 0.45um, 47mm nitrocellulose membrane (Pall Corporation, Ann Arbor, MI), placed on a 50mm petri dish containing mEI agar (BD Bioscience, Sparks, MD), and incubated at 41ºC for 24 hours. Cores of sediment from an individual station were mixed using a sterile spatula, 10 grams of sediment was blended with 200mL of phosphate buffer saline, and the container shaken vigorously for two minutes followed by settling for 1 minute. Sediment supernatant was filtered through a 0.45um, 47mm nitrocellulose membrane, placed on mEI agar, and incubated at 41°C for 24 hours. Colonies with raised blue halos (≥0.5 mm) were considered enterococci. Isolates were picked into 3mL of brain-heart infusion broth (BHIB) and incubated for 24 hours at 37°C .

Enterococci Suspension: Sediment containing enterococci was suspended as a slurry in 6L of sterile coastal water and sampled immediately for enterococcal counts. The water was sampled at 1, 2, 4, 8, 24, and 48 hours.

Organic Matter : 15g wet sediment sample were added to 120mL of sterile water, shaken vigorously for two minutes, and the supernatant filtered through a 4.25cm, 934AH grade, glass fiber filter (Reeve Angel, Clifton, New Jersey). 120mL of water was added two additional times and filtered to collect the remainder of the organic matter. After filtering, sediment was dried and weight was recorded. Glass fiber filter paper containing the organic matter was dried, weighed, and transferred to a 450°C muffle furnace for 5h. The ash weight was measured and recorded. The weight of organic matter was calculated as follows: Dry weight – Ash weight = Weight of Organic matter

PCR Template Preparation: Enterococcal BHIB cultures from each isolate (1mL) was pipetted into a sterile Nunc 96 DeepWell plates. Plates were centrifuged at 1600 X g, washed 2X with 250µl sterile water, cells resuspended in 250µl sterile water, and frozen at -20°C for PCR analysis.

PCR: 10 µl whole cell PCR reactions were conducted using 1µl washed cells, 1X PCR buffer (New England Biolabs, Beverly, MA.), 2.5 mM MgCl2, 250µm of dNTPs (NEB), 3µM each 30µM primer (REP1R and REP2I), 1X loading dye buffer, 0.5U/10 µl Taq polymerase(NEB). Thermal cycler protocol: Initial Denaturing: 95°C for 2 min; 35 Cycles of: 94°C for 3 s, 92°C for 30 s, 50°C for 60 s, 65°C for 8 min; Final annealing step 65°C for 8 min. PCR products were separated on using a 1.5% agarose gel for 6 hours at 55 volts in a 0.5X TAE buffer, stained in 0.1% EtBr for 20 minutes, and analyzed using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium).

Figure 1.

Table 1. Comparison of enterococcal counts and organic matter

Station 10	#EN/100mL	Org Matter (g)	Station 8	#EN/100mL	Org Matter (g)
5/3/2005	**940**	**0.0574**	**5/3/2005**	**303**	**0.0276**
5/31/2005	88	0.0057	5/31/2005	13	0.001
6/13/2005	**110**	**0.0136**	6/13/2005	0	0.0019
6/21/2005	21	0.0033	6/21/2005	11	0.0012
7/12/2005	39	0.0029	7/12/2005	19	0.0009
7/19/2005	39	0.0015	7/19/2005	0	0.0014
7/25/2005	36	0.0054	7/25/2005	103	0.0025
8/1/2005	43	0.0081	8/1/2005	4	0.0013

Table 2: Enterococci counts following suspension of sediment in marine water

Time (Hour)	0.083 hr	1 hr	2 hrs	4 hrs	8 hrs	24 hrs	48 hrs
CFU/100mL	38	25	17	17	10	9	1

Table 3: Jackknife analysis of sediment and water enterococcal fingerprints

Total: Jackknife (Maximum similarities) 704 Water isolates to 704 Sediment isolates

	Water	Sediment
Water	65.9	37.5
Sediment	34.1	62.5

5/31/2005 (Calm) Jackknife (Maximum similarities) 84 Water isolates to 84 Sediment isolates

	Water	Sediment
Water	71.1	44
Sediment	28.9	56

7/19/2005 (Rough) Jackknife (Maximum similarities) 58 Water isolates to 58 Sediment isolates

	Water	Sediment
Water	54.3	47.8
Sediment	45.7	52.2

Results & Discussion

Wave action along the Mississippi coast readily suspends the upper, fluffy sediment layer releasing enterococci (EN) into the water column. During calm wave conditions, we observed low bacterial levels (1.0 – 227 CFU/100mL) in the water, and higher counts were observed in the sediment; 10 – 351 CFU/100mL during rough wave conditions. Rough wave conditions (>12" wave height) cause continuous resuspension of the sediment. Mississippi coasts sediments and water contain high organic content due to artificial beach overlying a natural coastal marsh, drainage culverts along the coastline, and the blockage of ocean currents by the barrier islands. Table 1 represents the relationship between EN counts and organic matter in sediment samples. Samples containing higher levels of organic matter were generally correlated with higher EN counts providing evidence that enterococci may be attaching to the organic content in the water and settling into the sediment. Resuspension of organic matter and sediment is caused by waves, humans, boats, and animals. Settling rates of sediment-associated enterococci (Table 2) were determined by resuspending contaminated sediment into a container of sterile gulf coast water. A 50% decrease in enterococci counts were observed after 2 hours with an approximately 100% decrease after 48 hours of settling. The container was not disturbed during sampling, but settling time may be variable in the natural coastal environment. Jackknife analysis of total sediment and water isolates using maximum similarities (Table 3) showed that isolates from each group are similar. Enterococci isolated during rough wave conditions show a greater similarity between water column and sediment, while calm condition isolates showed less similarity.

Conclusions

- Enterococci attached to upper layer sediments are readily resuspended in the water column.
- Higher levels of organic matter appear to correlate with higher enterococci counts.
- Sediment-associated enterococci may persist in the water column for 48 hours before settling.
- Jackknife analysis demonstrates differences in sediment and water isolates.
- During rough wave conditions, sediment and water enterococcal isolates show greater similarity than during calm conditions.

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